

## Patent claims

1. A process for the preparation of a ketocarotenoid by culturing a genetically modified, nonhuman organism, which in comparison with the wild-type has a modified ketolase activity and a modified  $\beta$ -cyclase activity, and the modified  $\beta$ -cyclase activity is caused by a  $\beta$ -cyclase comprising the amino acid sequence SEQ. ID. NO. 2 or a sequence derived from this sequence by substitution, insertion or deletion of amino acids, which has an identity of at least 70% at the amino acid level with the sequence SEQ. ID. NO. 2.
2. The process according to claim 1, wherein a nonhuman organism is used which, as the wild-type, already has a ketolase activity, and the genetic modification causes an increase in the ketolase activity in comparison with the wild-type.
3. The process according to claim 2, wherein for increasing the ketolase activity the gene expression of a nucleic acid encoding a ketolase is increased compared to the wild-type.
4. The process according to claim 3, wherein for increasing the gene expression a nucleic acid which encodes a ketolase is inserted into the organism.
5. The process according to claim 4, wherein, as a nucleic acid encoding a ketolase, a nucleic acid is inserted which encodes a ketolase comprising the amino acid sequence SEQ ID NO: 4 or a sequence derived from this sequence by substitution, insertion or deletion of amino acids, which has an identity of at least 70% at the amino acid level with the sequence SEQ ID NO: 4.
6. The process according to claim 1, wherein a nonhuman organism is used which, as the wild-type, has no ketolase activity and the genetic modification causes a ketolase activity in comparison with the wild-type.
7. The process according to claim 6, wherein a genetically modified organism is used which transgenically expresses a ketolase.

8. The process according to claim 6 or 7, wherein, for causing the gene expression, a nucleic acid which encodes a ketolase is inserted into the organism.
9. The process according to claim 8, wherein a nucleic acid is inserted encoding a ketolase comprising the amino acid sequence SEQ. ID. NO. 4 or a sequence derived from this sequence by substitution, insertion or deletion of amino acids, which has an identity of at least 70% at the amino acid level with the sequence SEQ. ID. NO. 4.
10. The process according to claim 5 or 9, wherein a nucleic acid comprising the sequence SEQ. ID. NO. 3 is inserted.
11. The process according to any one of claims 1 to 10, wherein an organism is used which, as the wild-type, already has a  $\beta$ -cyclase activity, and the genetic modification causes an increase in the  $\beta$ -cyclase activity in comparison with the wild-type.
12. The process according to claim 11, wherein for increasing the  $\beta$ -cyclase activity the gene expression of a nucleic acid encoding a  $\beta$ -cyclase comprising the amino acid sequence SEQ. ID. NO. 2 or a sequence derived from this sequence by substitution, insertion or deletion of amino acids, which has an identity of at least 70% at the amino acid level with the sequence SEQ. ID. NO. 2, is increased compared to the wild-type.
13. The process according to claim 12, wherein for increasing the gene expression a nucleic acid is inserted into the organism, which encodes a  $\beta$ -cyclase comprising the amino acid sequence SEQ. ID. NO. 2 or a sequence derived from this sequence by substitution, insertion or deletion of amino acids, which has an identity of at least 70% at the amino acid level with the sequence SEQ. ID. NO. 2.
14. The process according to any one of claims 1 to 10, wherein an organism is used which, as the wild-type, has no  $\beta$ -cyclase activity and the genetic modification causes a  $\beta$ -cyclase activity in comparison with the wild-type.

15. The process according to claim 14, wherein a genetically modified organism is used which transgenically expresses a  $\beta$ -cyclase comprising the amino acid sequence SEQ. ID. NO. 2 or a sequence derived from this sequence by substitution, insertion or deletion of amino acids, which has an identity of at least 70% at the amino acid level with the sequence SEQ. ID. NO. 2.
16. The process according to claim 14 or 15, wherein for causing the gene expression a nucleic acid is inserted into the organism which encodes a  $\beta$ -cyclase comprising the amino acid sequence SEQ. ID. NO. 2 or a sequence derived from this sequence by substitution, insertion or deletion of amino acids, which has an identity of at least 70% at the amino acid level with the sequence SEQ. ID. NO. 2.
17. The process according to claim 13 or 16, wherein a nucleic acid comprising the sequence SEQ. ID. NO. 1 is inserted.
18. The process according to any one of claims 1 to 17, wherein the nonhuman organism additionally has an increased or caused hydroxylase activity compared to the wild-type.
19. The process according to claim 18, wherein for the additional increasing or causing of the hydroxylase activity, the gene expression of a nucleic acid encoding a hydroxylase is increased or caused compared to the wild-type.
20. The process according to claim 19, wherein for increasing or causing the gene expression a nucleic acid encoding a hydroxylase is inserted into the organism.
21. The process according to claim 20, wherein, as a nucleic acid encoding a hydroxylase, a nucleic acid is inserted which encodes a hydroxylase comprising the amino acid sequence SEQ ID NO: 6 or a sequence derived from this sequence by substitution, insertion or deletion of amino acids, which has an identity of at least 70% at the amino acid level with the sequence SEQ ID NO: 6.
22. The process according to claim 21, wherein a nucleic acid comprising the sequence SEQ ID NO: 5 is inserted.

23. The process according to any one of claims 1 to 22, wherein the organism additionally, compared to the wild-type, has an increased or caused activity of at least one of the activities selected from the group consisting of HMG-CoA reductase activity, (E)-4-hydroxy-3-methylbut-2-enyl diphosphate reductase activity, 1-deoxy-D-xylose 5-phosphate synthase activity, 1-deoxy-D-xylose 5-phosphate reductoisomerase activity, isopentenyl diphosphate  $\Delta$ -isomerase activity, geranyl diphosphate synthase activity, farnesyl diphosphate synthase activity, geranylgeranyl diphosphate synthase activity, phytoene synthase activity, phytoene desaturase activity, zeta-carotene desaturase activity, crtISO activity, FtsZ activity and MinD activity.
24. The process according to claim 23, wherein for the additional increasing or causing of at least one of the activities, the gene expression of at least one nucleic acid selected from the group consisting of a nucleic acid encoding an HMG-CoA reductase, a nucleic acid encoding an (E)-4-hydroxy-3-methylbut-2-enyl diphosphate reductase, a nucleic acid encoding a 1-deoxy-D-xylose 5-phosphate synthase, a nucleic acid encoding a 1-deoxy-D-xylose 5-phosphate reductoisomerase, a nucleic acid encoding an isopentenyl diphosphate  $\Delta$ -isomerase, a nucleic acid encoding a geranyl diphosphate synthase, a nucleic acid encoding a farnesyl diphosphate synthase, a nucleic acid encoding a geranylgeranyl diphosphate synthase, a nucleic acid encoding a phytoene synthase, a nucleic acid encoding a phytoene desaturase, a nucleic acid encoding a zeta-carotene desaturase, a nucleic acid encoding a crtISO protein, a nucleic acid encoding an FtsZ protein and a nucleic acid encoding an MinD protein is increased compared to the wild-type.
25. The process according to claim 24, wherein for the increasing or causing of the gene expression of at least one of the nucleic acids, at least one nucleic acid selected from the group consisting of a nucleic acid encoding an HMG-CoA reductase, a nucleic acid encoding an (E)-4-hydroxy-3-methylbut-2-enyl diphosphate reductase, a nucleic acid encoding a 1-deoxy-D-xylose 5-phosphate synthase, a nucleic acid encoding a 1-deoxy-D-xylose 5-phosphate reductoisomerase, a nucleic acid encoding an isopentenyl diphosphate  $\Delta$ -isomerase, a nucleic acid encoding a geranyl diphosphate synthase, a nucleic acid encoding a farnesyl diphosphate synthase, a nucleic acid encoding a

geranylgeranyl diphosphate synthase, a nucleic acid encoding a phytoene synthase, a nucleic acid encoding a phytoene desaturase, a nucleic acid encoding a zeta-carotene desaturase, a nucleic acid encoding a crtISO protein, a nucleic acid encoding an FtsZ protein and a nucleic acid encoding an MinD protein is  
5 inserted into the nonhuman organism.

26. The process according to claim 25, wherein, as the nucleic acid encoding an HMG-CoA reductase, a nucleic acid is inserted which encodes an HMG-CoA reductase comprising the amino acid sequence SEQ ID NO: 8 or a sequence derived from  
10 this sequence by substitution, insertion or deletion of amino acids, which has an identity of at least 20% at the amino acid level with the sequence SEQ ID NO: 8.

27. The process according to claim 26, wherein a nucleic acid comprising the sequence SEQ ID NO: 7 is inserted.  
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28. The process according to claim 25, wherein as a nucleic acid encoding an (E)-4-hydroxy-3-methylbut-2-enyl diphosphate reductase, a nucleic acid is inserted which encodes an (E)-4-hydroxy-3-methylbut-2-enyl diphosphate reductase comprising the amino acid sequence SEQ ID NO: 10 or a sequence derived from this  
20 sequence by substitution, insertion or deletion of amino acids, which has an identity of at least 20% at the amino acid level with the sequence SEQ ID NO: 10.

29. The process according to claim 28, wherein a nucleic acid comprising the sequence SEQ ID NO: 9 is inserted.  
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30. The process according to claim 25, wherein, as a nucleic acid encoding a 1-deoxy-D-xylose 5-phosphate synthase, a nucleic acid is inserted which encodes a 1-deoxy-D-xylose 5-phosphate synthase comprising the amino acid sequence SEQ ID NO: 12 or a sequence derived from this sequence by substitution, insertion or  
30 deletion of amino acids, which has an identity of at least 20% at the amino acid level with the sequence SEQ ID NO: 12.

31. The process according to claim 30, wherein a nucleic acid comprising the sequence SEQ ID NO: 11 is inserted.  
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32. The process according to claim 25, wherein as a nucleic acid encoding a 1-deoxy-D-xylose 5-phosphate reductoisomerase, a nucleic acid is inserted which encodes a 1-deoxy-D-xylose 5-phosphate reductoisomerase comprising the amino acid sequence SEQ ID NO: 14 or a sequence derived from this sequence by substitution, insertion or deletion of amino acids, which has an identity of at least 20% at the amino acid level with the sequence SEQ ID NO: 14.
33. The process according to claim 32, wherein a nucleic acid comprising the sequence SEQ ID NO: 13 is inserted.
34. The process according to claim 25, wherein as a nucleic acid encoding an isopentenyl diphosphate  $\Delta$ -isomerase, a nucleic acid is inserted which encodes an isopentenyl diphosphate  $\Delta$ -isomerase, comprising the amino acid sequence SEQ ID NO: 16 or a sequence derived from this sequence by substitution, insertion or deletion of amino acids, which has an identity of at least 20% at the amino acid level with the sequence SEQ ID NO: 16.
35. The process according to claim 34, wherein a nucleic acid comprising the sequence SEQ ID NO: 15 is inserted.
36. The process according to claim 25, wherein as a nucleic acid encoding a geranyl diphosphate synthase, a nucleic acid is inserted which encodes a geranyl diphosphate synthase comprising the amino acid sequence SEQ ID NO: 18 or a sequence derived from this sequence by substitution, insertion or deletion of amino acids, which has an identity of at least 20% at the amino acid level with the sequence SEQ ID NO: 18.
37. The process according to claim 36, wherein a nucleic acid comprising the sequence SEQ ID NO: 17 is inserted.
38. The process according to claim 25, wherein as a nucleic acid encoding a farnesyl diphosphate synthase, a nucleic acid is inserted which encodes a farnesyl diphosphate synthase comprising the amino acid sequence SEQ ID NO: 20 or a sequence derived from this sequence by substitution, insertion or deletion of amino acids, which has an identity of at least 20% at the amino acid level with the

sequence SEQ ID NO: 20.

39. The process according to claim 38, wherein a nucleic acid comprising the sequence SEQ ID NO: 19 is inserted.

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40. The process according to claim 25, wherein as a nucleic acid encoding a geranylgeranyl diphosphate synthase, a nucleic acid is inserted which encodes a geranylgeranyl diphosphate synthase comprising the amino acid sequence SEQ ID NO: 22 or a sequence derived from this sequence by substitution, insertion or deletion of amino acids, which has an identity of at least 20% at the amino acid level with the sequence SEQ ID NO: 22.

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41. The process according to claim 40, wherein a nucleic acid comprising the sequence SEQ ID NO: 21 is inserted.

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42. The process according to claim 25, wherein as a nucleic acid encoding a phytoene synthase, a nucleic acid is inserted which encodes a phytoene synthase comprising the amino acid sequence SEQ ID NO: 24 or a sequence derived from this sequence by substitution, insertion or deletion of amino acids, which has an identity of at least 20% at the amino acid level with the sequence SEQ ID NO: 24.

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43. The process according to claim 42, wherein a nucleic acid comprising the sequence SEQ ID NO: 23 is inserted.

44. The process according to claim 25, wherein as a nucleic acid encoding a phytoene desaturase, a nucleic acid is inserted which encodes a phytoene desaturase comprising the amino acid sequence SEQ ID NO: 26 or a sequence derived from this sequence by substitution, insertion or deletion of amino acids, which has an identity of at least 20% at the amino acid level with the sequence SEQ ID NO: 26.

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45. The process according to claim 44, wherein a nucleic acid comprising the sequence SEQ ID NO: 25 is inserted.

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46. The process according to claim 25, wherein as a nucleic acid encoding a zeta-carotene desaturase, a nucleic acid is inserted which encodes a zeta-carotene

desaturase comprising the amino acid sequence SEQ ID NO: 28 or a sequence derived from this sequence by substitution, insertion or deletion of amino acids, which has an identity of at least 20% at the amino acid level with the sequence SEQ ID NO: 28.

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47. The process according to claim 46, wherein a nucleic acid comprising the sequence SEQ ID NO: 27 is inserted.

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48. The process according to claim 25, wherein as a nucleic acid encoding a crtISO protein, a nucleic acid is inserted which encodes a crtISO protein comprising the amino acid sequence SEQ ID NO: 30 or a sequence derived from this sequence by substitution, insertion or deletion of amino acids, which has an identity of at least 20% at the amino acid level with the sequence SEQ ID NO: 30.

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49. The process according to claim 48, wherein a nucleic acid comprising the sequence SEQ ID NO: 29 is inserted.

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50. The process according to claim 25, wherein as a nucleic acid encoding an FtsZ protein, a nucleic acid is inserted which encodes an FtsZ protein comprising the amino acid sequence SEQ ID NO: 32 or a sequence derived from this sequence by substitution, insertion or deletion of amino acids, which has an identity of at least 20% at the amino acid level with the sequence SEQ ID NO: 32.

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51. The process according to claim 50, wherein a nucleic acid comprising the sequence SEQ ID NO: 31 is inserted.

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52. The process according to claim 25, wherein as a nucleic acid encoding an MinD protein, a nucleic acid is inserted which encodes an MinD protein comprising the amino acid sequence SEQ ID NO: 34 or a sequence derived from this sequence by substitution, insertion or deletion of amino acids, which has an identity of at least 20% at the amino acid level with the sequence SEQ ID NO: 34.

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53. The process according to claim 52, wherein a nucleic acid comprising the sequence SEQ ID NO: 33 is inserted.



54. The process according to any one of claims 1 to 53, wherein after the culturing the genetically modified organism is harvested and the ketocarotenoid is subsequently isolated from the organism.
- 5 55. The process according to any one of claims 1 to 54, wherein, as the organism, an organism is used which, as the starting organism, naturally or by genetic complementation or reregulation of metabolic pathways is in the position to produce carotenoids.
- 10 56. The process according to any one of claims 1 to 55, wherein the organism used is a microorganism or plant.
57. The process according to claim 56, wherein the microorganism used is a bacterium, yeast, alga or fungus.
- 15 58. The process according to claim 57, wherein the microorganism is selected from the group consisting of *Escherichia*, *Erwinia*, *Agrobacterium*, *Flavobacterium*, *Alcaligenes*, *Paracoccus*, *Nostoc*, a cyanobacterium of the genus *Synechocystis*, *Candida*, *Saccharomyces*, *Hansenula*, *Phaffia*, *Pichia*, *Aspergillus*, *Trichoderma*,  
20 *Ashbya*, *Neurospora*, *Blakeslea*, *Phycomyces*, *Fusarium*, *Haematococcus*, *Phaedactylum tricornatum*, *Volvox* or *Dunaliella*.
59. The process according to claim 56, wherein the organism used is a plant.
- 25 60. The process according to claim 59, wherein the plant used is a plant selected from the families Amaranthaceae, Amaryllidaceae, Apocynaceae, Asteraceae, Balsaminaceae, Begoniaceae, Berberidaceae, Brassicaceae, Cannabaceae, Caprifoliaceae, Caryophyllaceae, Chenopodiaceae, Compositae, Cucurbitaceae, Cruciferae, Euphorbiaceae, Fabaceae, Gentianaceae, Geraniaceae, Graminae, 30 Illiaceae, Labiatae, Lamiaceae, Leguminosae, Liliaceae, Linaceae, Lobeliaceae, Malvaceae, Oleaceae, Orchidaceae, Papaveraceae, Plumbaginaceae, Poaceae, Polemoniaceae, Primulaceae, Ranunculaceae, Rosaceae, Rubiaceae, Scrophulariaceae, Solanaceae, Tropaeolaceae, Umbelliferae, Verbanaceae, Vitaceae or Violaceae.
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61. The process according to claim 60, wherein the plant used is a plant selected from the plant genera Marigold, *Tagetes erecta*, *Tagetes patula*, *Acacia*, *Aconitum*, *Adonis*, *Arnica*, *Aquilegia*, *Aster*, *Astragalus*, *Bignonia*, *Calendula*, *Caltha*, *Campanula*, *Canna*, *Centaurea*, *Cheiranthus*, *Chrysanthemum*, *Citrus*, *Crepis*,  
5 *Crocus*, *Curcubita*, *Cytisus*, *Delonia*, *Delphinium*, *Dianthus*, *Dimorphotheca*, *Doronicum*, *Eschscholtzia*, *Forsythia*, *Fremontia*, *Gazania*, *Gelsemium*, *Genista*, *Gentiana*, *Geranium*, *Gerbera*, *Geum*, *Grevillea*, *Helenium*, *Helianthus*, *Hepatica*, *Heracleum*, *Hibiscus*, *Heliopsis*, *Hypericum*, *Hypochoeris*, *Impatiens*, *Iris*, *Jacaranda*, *Kerria*, *Laburnum*, *Lathyrus*, *Leontodon*, *Lilium*, *Linum*, *Lotus*,  
10 *Lycopersicon*, *Lysimachia*, *Marattia*, *Medicago*, *Mimulus*, *Narcissus*, *Oenothera*, *Osmanthus*, *Petunia*, *Photinia*, *Physalis*, *Phyteuma*, *Potentilla*, *Pyracantha*, *Ranunculus*, *Rhododendron*, *Rosa*, *Rudbeckia*, *Senecio*, *Silene*, *Silphium*, *Sinapsis*, *Sorbus*, *Spartium*, *Tecoma*, *Torenia*, *Tragopogon*, *Trollius*, *Tropaeolum*, *Tulipa*, *Tussilago*, *Ulex*, *Viola* or *Zinnia*.
- 15 62. The process according to any one of claims 1 to 61, wherein the ketocarotenoid is selected from the group consisting of astaxanthin, canthaxanthin, echinenone, 3-hydroxyechinenone, 3'-hydroxyechinenone, adonirubin and adonixanthin.
- 20 63. A genetically modified, nonhuman organism, where the genetic modification,
- A for the case where the wild-type organism already has a ketolase activity, increases the activity of a ketolase compared to the wild-type and
- 25 B for the case where the wild-type organism has no ketolase activity, causes the activity of a ketolase compared to the wild-type,
- and where the genetic modification,
- 30 C for the case where the wild-type organism already has a  $\beta$ -cyclase activity, increases the activity of a  $\beta$ -cyclase compared to the wild-type and
- D for the case where the wild-type organism has no  $\beta$ -cyclase activity, causes the activity of a  $\beta$ -cyclase compared to the wild-type

and the  $\beta$ -cyclase activity increased according to C or caused according to D is caused by a  $\beta$ -cyclase comprising the amino acid sequence SEQ. ID. NO. 2 or a sequence derived from this sequence by substitution, insertion or deletion of amino acids, which has an identity of at least 70% at the amino acid level with the sequence SEQ. ID. NO. 2.

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64. The genetically modified organism according to claim 63, wherein, as the starting organism, it is in the position naturally or by genetic complementation to produce a carotenoid.

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65. The genetically modified organism according to either of claims 63 or 64, selected from the group consisting of a microorganism or plant.

66. The genetically modified organism according to claim 65, wherein the microorganism is selected from the group consisting of a bacterium, yeast, alga or fungus.

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67. The genetically modified organism according to claim 66, wherein the microorganism is selected from the group consisting of *Escherichia*, *Erwinia*, *Agrobacterium*, *Flavobacterium*, *Alcaligenes*, *Paracoccus*, *Nostoc*, a cyanobacterium of the genus *Synechocystis*, *Candida*, *Saccharomyces*, *Hansenula*, *Pichia*, *Aspergillus*, *Trichoderma*, *Ashbya*, *Neurospora*, *Blakeslea*, *Phycomyces*, *Fusarium*, *Haematococcus*, *Phaedactylum tricornatum*, *Volvox* or *Dunaliella*.

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68. The genetically modified plant according to claim 65, wherein the plant is selected from the families Amaranthaceae, Amaryllidaceae, Apocynaceae, Asteraceae, Balsaminaceae, Begoniaceae, Berberidaceae, Brassicaceae, Cannabaceae, Caprifoliaceae, Caryophyllaceae, Chenopodiaceae, Compositae, Cucurbitaceae, Cruciferae, Euphorbiaceae, Fabaceae, Gentianaceae, Geraniaceae, Graminae, Illiaceae, Labiatae, Lamiaceae, Leguminosae, Liliaceae, Linaceae, Lobeliaceae, Malvaceae, Oleaceae, Orchidaceae, Papaveraceae, Plumbaginaceae, Poaceae, Polemoniaceae, Primulaceae, Ranunculaceae, Rosaceae, Rubiaceae, Scrophulariaceae, Solanaceae, Tropaeolaceae, Umbelliferae, Verbanaceae,

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Vitaceae and Violaceae used.

69. The genetically modified plant according to claim 68, wherein the plant is selected from the plant genera Marigold, Tagetes erecta, Tagetes patula, Acacia, Aconitum, Adonis, Arnica, Aquilegia, Aster, Astragalus, Bignonia, Calendula, Caltha, Campanula, Canna, Centaurea, Cheiranthus, Chrysanthemum, Citrus, Crepis, Crocus, Curcurbita, Cytisus, Delonia, Delphinium, Dianthus, Dimorphotheca, Doronicum, Eschscholtzia, Forsythia, Fremontia, Gazania, Gelsemium, Genista, Gentiana, Geranium, Gerbera, Geum, Grevillea, Helenium, Helianthus, Hepatica, Heracleum, Hisbiscus, Heliopsis, Hypericum, Hypochoeris, Impatiens, Iris, Jacaranda, Kerria, Laburnum, Lathyrus, Leontodon, Lilium, Linum, Lotus, Lycopersicon, Lysimachia, Maratia, Medicago, Mimulus, Narcissus, Oenothera, Osmanthus, Petunia, Photinia, Physalis, Phyteuma, Potentilla, Pyracantha, Ranunculus, Rhododendron, Rosa, Rudbeckia, Senecio, Silene, Silphium, Sinapsis, Sorbus, Spartium, Tecoma, Torenia, Tragopogon, Trollius, Tropaeolum, Tulipa, Tussilago, Ulex, Viola or Zinnia used.
70. The use of the genetically modified organism according to any one of claims 63 to 69 as a feed or food.
71. The use of the genetically modified organism according to any one of claims 63 to 69 for the production of a ketocarotenoid-containing extract or for the production of a feed supplement or food supplement.